4th Asian Academic Society International Conference (AASIC) 2016

Globalizing Asia: Integrating Science, Technology and Humanities for Future Growth and Development

HEA-OR-012

POLYMORPHISM OF FPN1 GENE PROMOTER -1355 G/C AS A RISK FACTOR OF IRON DEFICIENCY ANEMIA IN PREGNANT WOMEN IN INDONESIA

Nor Istiqomah[1], **Sarah Safira Umarghanies[2],** Arta Farmawati[3], Ahmad Hamim Sadewa[3], Yuliana Heri Soesilo[4], Kusumadewi Eka Damayanti[4], Dono Indarto[4]

[1]*Faculty of Health Sciences, Universitas Pekalongan, Pekalongan, Indonesia*
[2]*Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[3]*Department of Biochemistry, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[4]*Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia*

Corresponding author's email: sarahsafira.umarghanies@ymail.com

According to WHO, the prevalence of anemia in pregnancy in Indonesia is 44.3%, it is higher than the worldwide prevalence (41.8%). Iron deficiency is a leading cause of anemia during pregnancy. Ferroportin (FPN1) is an iron exporter protein which is responsible on absorbing and releasing iron in order to reproduce new erythocytes. The variant of FPN1-1355 G/C in promoter region enhances overexpression of ferroportin and iron export, which are causing elevated cellular iron needs and thus leading to overexpression of soluble transferrin receptor (sTfR) while decreasing hemoglobin (Hb) level and erythrocyte indices. As the manifestation, iron deficiency anemia (IDA) occurs. This research was a case and control study aimed to analyze the frequency of FPN1-1355 G/C polymorphism as a risk factor of IDA in pregnant women in Indonesia. Blood samples were taken from 26 pregnant women with anemia and 48 without anemia. FPN1-1355 G/C polymorphism were determined by PCR-RFLP method while sTfR and ferritin level were measured by ELISA. Hemoglobin, erythocyte indices, and sTfR level were compared among genotype group, then analyzed by independent t-test and one way ANOVA. Pearson test was conducted to analyze correlation between level of hemoglobin and ferritin in pregnant women ($p<0.05$). As the results, the frequency of FPN1-1355 G/C polymorphism in anemic pregnant women with IDA and non IDA were 100% and 95.2%, respectively ($p=1.000$; OR=0.800; 95%CI= 0.658-0.973). The mean of Hb level and erythrocyte indices in subject carrying C allele were lower than subjects carrying only G allele, although Hb level was not significantly different ($p>0.05$). The sTfR level in subject carrying C allele was higher than subjects carrying only G allele ($p<0.05$). As a conclusion, FPN1 gene promoter -1355 G/C polymorphism was not a risk factor for anemia, but it was a risk factor for IDA in pregnant women.

Keywords: ferroportin (FPN1) -1355 G/C polymorphism, iron deficiency anemia, soluble transferin receptor (sTfR), ferritin.

1. INTRODUCTION

Iron deficiency is the most common type of anemia worldwide, affecting both developed and developing countries. Pregnant women are one of the vulnerable group susceptible from anemia due to physiologic process (WHO, 2008). Anemia in pregnant women could lead to premature labor, low birth weight newborn, infection, antepartum and postpartum hemorrhage, or even death

(Knutson, 2010; Nemeth, 2010). Prevalence of anemia in pregnancy was still high in Surakarta, about 53.4%, regardless of higher iron supplementation program coverage (80%) (Ministry of Health Republic of Indonesia, 2011).

On iron homeostasis, ferroportin is a transporter protein coded by FPN1 gene which is responsible on iron distribution throughout body (Nemeth, 2010). Previous research found no variant or polymorphism of ferroportin Q248H gene in pregnant women population in Surakarta (Farmawati et al., 2012). Nevertheless, that iron transporter gene was still suspected as the cause of iron absorption disturbance, considering how importance ferroportin is for iron homeostasis. So this research aimed to analyze the polymorphism of ferroportin gene in promoter region, especially in -1355 G/C, as a risk factor of IDA in pregnant women.

2. RESEARCH METHODOLOGY

An analytical case-control research was held on May 2012 to January 2013. The subjects were pregnant women attending Primary Health Care (PHC) in seven districts in Surakarta, Central Java Province, Indonesia. Samples were collected by consecutive sampling. The inclusion criterias were pregnant women aged 18-35 years old at 10-25 weeks of gestational age (second and third trimester) and willing to sign informed consent. The exclusion criterias were if subject reported herself had any chronic diseases (tuberculosis, diabetes mellitus, malaria, heart disease, hepatitis) which could interfere erythropoiesis, or had severe anemia (Hb below 7 g/dL).

At first, 120 pregnant women were signing the informed consents, but 46 subjects were not eligible. Thus, measurement of Hb level (by cyanmethemoglobin method) and erythrocyte indices were done on 74 subjects for grouping. There were 24 subjects for anemia group (Hb <11 g/dL) and 48 subjects for non-anemia group (Hb > 11g/dL). For specific purposes, anemia group was divided again into IDA group and non-IDA group based on level of Hb (<11 g/dL) and erythrocyte indices (mean corpuscular volume/MCV <80 fl and/or mean corpuscular hemoglobin concentration/MCHC <31%) (Ministry of Health Republic of Indonesia, 2011). From 26 anemia subjects, 5 were included in IDA group and 21 in non-IDA group.

Deoxyribose nucleic acid (DNA) was then measured by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Ferritin level was measured by enzyme-linked immunosorbent assay (ELISA) method using DRG® Ferritin (EIA-1872) kit, and so did sTfR (using Quantikine IVD® kit). Genomes of DNA were isolated from blood samples by Promega® isolation kit. Moreover, DNA genomes were amplified by PCR method using Master Mix Go Taq Green Promega® kit. PCR products were then cut off by Bfal enzyme (MBI Fermentas®). The electrophoresis of DNA amplifications was done in 2% agarose gel on Tris-Boric Acid-EDTA buffer.

Collected data was analyzed by SPSS 19 program, a $p<0.05$ was considered as significant. The mean of of Hb, sTfR, dan ferritin between IDA group and non-IDA group were analyzed by independent sample t-test. The mean of Hb, sTfR, and ferritin among genotype group were analyzed by one-way ANOVA. Genotype frequency of each group was analyzed by one-way ANOVA, then the OR was determined. Correlation between level of hemoglobin and ferritin was analyzed by Pearson test.

3. RESULTS

From 74 samples, the mean age of subjects was 26,5±4.6 years old, with the youngest was 19 and oldest was 35. The mean of gestational age was 16.4±4.2 weeks, with the minimum was 10 weeks and maximum was 25 weeks. Subjects were divided into anemia group and non-anemia

group based on their Hb level. 48 subjects (61.5%) were included in non-anemia group while 26 others were in anemia group (**Table 1**). Anemia group was then divided again into IDA group and non-IDA group based on Hb level and erythrocyte indices (**Table 2**).

Table 1. Characteristic of Anemia and Non-Anemia Group

Characteristic	Value (mean±SD)		p (CI 95%)
	Anemia (n=26)	Non-anemia (n=48)	
Maternal age (year)	25.923±4.454	26.604±4.676	0.545 (-2.914;1.552)*
Gestational age (week)	18.308±4.576	15.250±3.449	0.002 (1.175;4.940)*

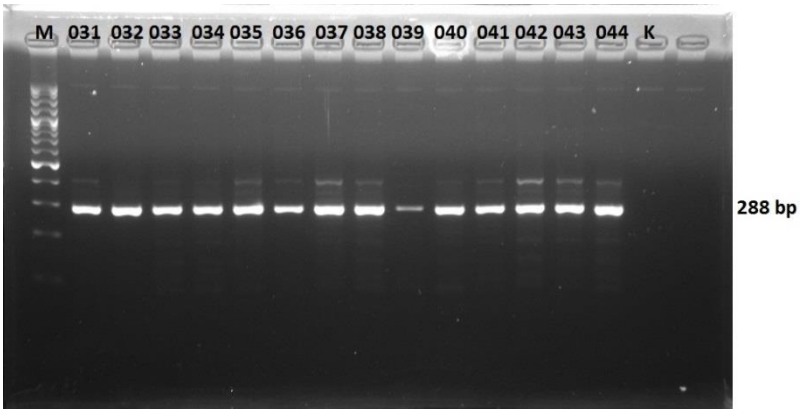
Notes: *independent sample t-test; SD= standard deviation; CI= confidence interval

Table 2. Characteristic of IDA and Non-IDA Group

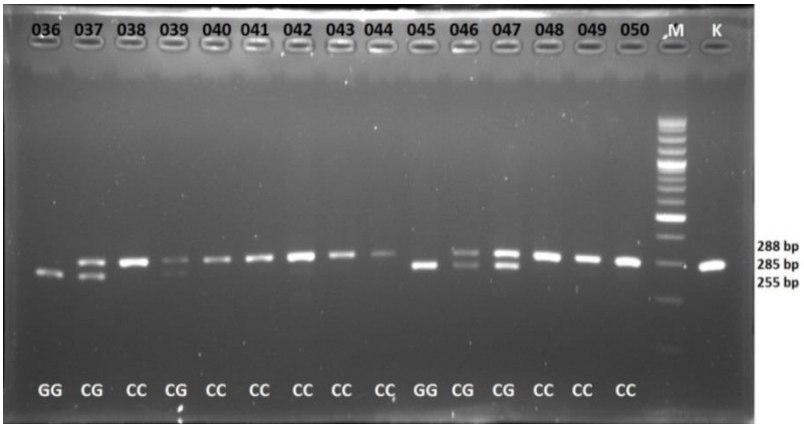
Characteristic	Value (mean±SD)		p (CI 95%)
	IDA (n=5)	Non-IDA (n=21)	
Maternal age (year)	23.800±3.962	26.429±4.501	0.243 (-7.163;1.906)*
Gestational age (week)	14.600 ± 5.727	19.190±3.919	0.041 (-8.980;-0.201)*
Hb (g/dL)	10.200±0.579	10.371±0.362	0.405 (-0.589; 0.246)*
MCV (fL)	69.940±4.475	88.509±4.545	0.000 (-23.226;-13.913)*
MCH (pg/sel)	22.200±2.019	29.386±1.910	0.000 (-9.166;-5.205)*
MCHC (g/dL)	31.270±0.920	33.195±1.387	0.034 (-2.831;-0.119)*

Note: *independent sample t-test; IDA= iron deficiency anemia; Hb= hemoglobin; MCV= mean corpuscular volume, MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration

Genotype investigation was done by PCR-RFLP method. The PCR product of FPN1 -1355 gene had size of 392 bp (**Picture 1**), which was then cut off by Bfal enzyme. C allele (variant) would be 285 bp in size while G allele (wild type) would be 255 and 30 bp (**Picture 2**).



Picture 1. PCR results of FPN1 -1355 gene, sample no. 31, 32, 33, 34, 35, 36, 37, 38, 41, 42, 43, and 44. Notes: K = control; M= marker; length of PCR product was 288 bp



Picture 2. PCR-RFLP results of FPN1 -1355 G/C, sample no. 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50. PCR products were digested by Bfal enzyme. GG genotype was showed in 255 and 30 bp band; GC in 285, 255, and 30 bp; and CC in 285 bp. Notes: K= control; M= marker; GG= wild type; CG= mutant heterozygote; CC= mutant homozygote

On this study, five pregnant women in IDA group were all having GC+CC genotype or carrying C allele. Meanwhile, GG genotype was not found in IDA group, so in this study C allele was considered as variant even though the frequency was higher than G allele. The OR of C allele for IDA incident was 1.600 (95%CI: 0.296-8.653) (**Table 3**).

Table 3. Genotype (CC, CG, GG) and allele (C, G) distribution of *FPN1* gene promoter -1355 in IDA and non-IDA group

Variable		IDA n=5	Non-IDA n=21	p	OR (CI 95%)
Genotype	CC	3 (60%)	10 (47.6%)	0.814	0.814
	CG	2 (40%)	10 (47.6%)		
	GG	0 (0%)	1 (4.8%)		
	GC + CC	5 (100%)	20 (95.2%)	1.000	0.800 (0.658-0.973)
Allele	C	8 (80%)	30 (71.4%)	0.710	1.600 (0.296-8.653)
	G	2 (20%)	12 (28.6%)		

The mean of Hb, MCV, MCH, and MCHC of C allele in IDA group showed lower level (Table 4) than non-IDA group, although Hb level was not statistically significant (p=0.401). It is well-known that Hb level is not a specific indicator for IDA. The mean of sTfR level in IDA group with C allele genotype (polymorphism) was significantly higher than non-IDA group either wild type (GG) or polymorphism (CG/CC) ($p<0.05$). Nevertheless, the mean of ferritin level was higher in subject carrying C allele than G allele (p=0.045), and non-IDA group's ferritin was lower than IDA group's.

Table 4. The mean difference of Hb, erythrocyte indices, sTfR, and ferritin between genotype groups among anemia subjects

Variable	Value (mean±SD)				
	IDA (n=5)		Non-IDA (n=17)		
	Wild type (n=0)	Polymorphism (n=5)	*Wild type* (n=1)	Polymorphism (n=20)	p
Hb (g/dL)		10.200±0.579	10.800	10.350±0.358	0.401*
MCV (fL)		69.940±4.475	97.500	88.060±4.157	0.000*
MCH (pg/cell)		22.200±2.019	33.200	29.195±1.742	0.000*
MCHC (g/dL)		31.720±0.920	34.100	33.150±1.406	0.088*
sTfR (nmol/L)		30.905±8.912	26.294	18.651±6.729 (n=18)	0.009*
Ferritin (ng/mL)		196.167±1.17	23.250	66.048±1.17	0.045*

Notes: *one way ANOVA; IDA= iron deficiency anemia; SD= standard deviation; Hb= hemoglobin; MCV= mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration; sTfR= soluble transferrin receptor

The investigation of ferritin level described iron storage within body. The mean of ferritin level was 48.5±46.1 ng/mL, with the minimum level was 1.2 ng/mL and maximum was 197.9 ng/mL. 26 subjects (63.4%) were having normal serum ferritin level (12-120 mg/mL), 9 subjects (22%) were low (<12 ng/mL), and 6 subjects (14.6%) were high (>120 ng/mL). It showed that most subjects had adequate iron storage within body.

4. DISCUSSION

FPN1 gene has promoter regions or transcription factor bindings (TFBs) which are responsible in transcriptional level of gene expression. It is known that untranslated region (5'-UTR) of FPN1 involves on iron homeostasis by regulating iron-responsive element (IRE). Post-transcriptionally, iron homeostasis is controlled by iron-regulatory protein (IRP1 and IRP2). Meanwhile, IRPs are protein-binding RNA in cytocol. The intracellular iron level regulates several proteins which are involved in iron homeostasis. Iron-regulatory protein will bind the IRE in 5' or 3' UTR of the mRNA which is coding that proteins. Both IRPs and IRE are responsible in connecting the sense and giving response to change the iron level within cell. It depends on where IRE is located, IRP binding would give the different effect on protein synthesis. From previous research in porphyria cutanea tarda (PCT) patients with iron metabolism disturbance, nucleotide transversion from G to C in 1355 position, upstreamly from ATG initiation (5'UTR -1355 G/C), was found 8 (34.8%) homozygous and 13 (56.5%) heterozygous (Hallendorff, 2008). According to HapMap Data, frequency of polymorphic allele of 5'UTR-FPN1-1355 G/C among Asia population was 10.4-16.2%. Other research mentioned that the polymorphism was 64% heterozygous in PCT subjects with iron metabolism disturbance (Panton, 2008).

Normally, cellular iron would be released from reticulocytes, duodenal enterocytes, placenta, hepatocytes, and spleen's macrophages through ferroportin transporter. Macrophage would release iron in eryhtrocyte catabolism. Meanwhile, bone marrow would picks up circulating iron for erythropoiesis. This condition goes in cycle to keep the iron homeostasis. Hepcidin as a regulator of ferroportin expression is stimulated by serum iron level, erythropoiesis activity, and inflammation (Nemeth, 2010; Pietrangelo, 2004; Cui et al., 2009). Furthermore, polymorphism of FPN1 could change the ferroportin expression and elevate the binding sensitivity to hepcidin.

Hepcidin binding on its binding-side in ferroportin surface leads to phosphorylation, internalization, and degradation of ferroportin by lysosome (Crichton, 2009)

On this research, all IDA subjects were having GC+CC genotype or carrying C allele. The OR of C allele for IDA incidence was 1.600 (95%CI:0.296-8.653), so that we could conclude that C allele was a risk factor for IDA. Subjects with C allele have more risk to develop IDA, about 1.6 times greater than G allele. C allele in FPN1 gene promoter -1355 5'UTR is known able to raise the translation of FPN1 gene, thus ferroportin expression is increasing. IRE located in FPN1 gene 5'UTR is functional on liver, intestines, and monocytes, so it is possible to response on iron manipulation freely from another promoter elements. Research on human indicated that mutation on FPN1-IRE could evoke deregulation of cellular iron export and lead to develop certain diseases (or as a modification factor of fenotype expression) such as hemochromatosis and IDA (Lymboussaki et al., 2003).

C allele in FPN1 -1355 G/C polymorphism generates FOXC1 transcription factor binds to IRE 5', thus inducing elevation of FPN1 gene translation (Hallendroff, 2008). Elevated ferroportin expression leads to raise in iron export. The increasing iron export would cause reduction of ferritin concentration and enhancement of cellular iron needs, thus leading to elevation of sTfR expression. Iron deficiency state disrupts the erythropoiesis process, and it is characterized by lower level of Hb, MCV, MCH, and MCHC. The statistically not significant result on allele frequency between IDA and non-IDA group probably occured because of the grouping method were based on Hb and erythrocyte indices only, which both had low sensitivity and specificity.

Significant result was merely showed on MCV, MCH, and sTfR level among genotype of IDA and non-IDA group ($p<0.05$). It is known that erythrocyte indices, and sTfR have higher sensitivity and specificity than Hb level in order to diagnose IDA (Akesson et al., 1998; Wians et al., 2001; Lopez-Sierra et al., 2012). Moreover, lower Hb, MCV, MCH, MCHC and higher sTfR in pregnant women with CG and CC genotype were in accordance with OR of C allele to IDA incident as high as 1.600, so that the C allele was a risk factor of IDA incidence.

Determination of C allele as a risk factor was then confirmed by Hb, erythrocyte indices, serum sTfR, and ferritin level to each genotype. Measurement among 26 pregnant women with anemia showed that only 5 subjects were having IDA, which meant that not all of subjects in anemia group were having iron deficiency anemia. On iron deficiency state, there is a raise in free porphyrin within erythrocytes, so that the enzyme containing iron on Hb, ferrochelatase, is inhibited. Protein synthesis in Hb is also inhibited, but probably there is a residual chain initiation. Ribonucleotide reductase (RR) is a non-heme iron protein which is the most sensitive to iron deficiency condition, that could induce inhibition of DNA synthesis. RR inhibition by hydroxyurea was manifested in macrocytic anemia (DNA synthesis inhibition), not a microcytic anemia, as the implementation of IDA (Kell, 2009).

Confirmation test to ferritin level revealed that most subjects had adequate iron storage. Nevertheless, ferritin is an acute phase protein and also a non specific iron storage indicator in patients with infection, inflammation, cancer, liver disease, and overactivity. If those conditions occur, ferritin level would raise and thus unable to measure iron storage proportionally because inflammation process would inhibit iron mobilization from its storage in reticuloendothelial cells (Kell, 2009; Lwanga, 2008; Van der Broek, 1998). Analysis found that Hb level and serum ferritin were having negative correlation with a very weak correlation power ($r=0.064$; $p=0.689$). This result meant that there will be a Hb reduction if there's a ferritin enhancement, vice versa. On this research, subjects with infection, inflammation, cancer, liver disease were not identified by specific

measurement during sample collection, thus the higher serum ferritin could be caused by those conditions.

Ferroportin is regulated post-translationally by hepcidin, a peptide hormone. Hepcidin as a major iron regulator protein is responsible in regulating iron release inhibitionally by binding with ferroportin and enhancing both internalization and degradation of ferroportin (Knutson, 2010; Wish, 2006). On inflammation, Kupffer cell in liver would release interleukin-6 (IL-6) which would increase hepcidin activity. This explains a reticuloendothelial blokage, where the iron storage cannot be released, represents in high serum ferritin whilst transferrin saturation is low (Wish, 2006). There is a lot of protein involves in iron homeostasis, thus it is important to elaborate the pathogenesys of IDA in pregnant women. On this research, the inflammation status, ferroportin level, and hepcidin serum were not measured, so those factors could interfere the result of this research.

This research explained that the genetic variant of FPN1 -1355 G/C in pregnant women did not increase the risk of anemia generally, but IDA specifically, which was reflected in low Hb and erythrocyte indices whilst sTfR level was high. Nevertheless, iron deficiency mentioned above was described as depletion of iron level for erythropoiesis or iron transportation disorder, despite the adequate iron storage (ferritin). This explanation was in accordance with the negative correlation results, reflected that the erythropoiesis disorder mentioned was not caused by low iron intake but iron export disturbance.

The higher non-IDA prevalence (80%) in pregnant women more than IDA (20%) found on this research revealed that the pathogenesys of anemia during pregnancy was more complex than an ineffective erythropoiesis caused by either iron or folat deficiency. Pregnancy state could elevate oxidative stress that could release proinflammatory cytokines such as interleukin-6 (IL-6), interleukin-1 (IL-1), gamma interferon (IF-γ), and alpha tumor necrosis factor (TNF-α). Those cytokines could activate janus kinase/signal transducer and activator of transcription pathway (JAK-STAT) so that the hepcidin synthesis is increasing. Hepcidin would inhibit the release of iron from macrophageal systems, thus leading to hypoferemia and iron restriction for erythropoiesis. This explains inflammation anemia, not IDA (Mackenzie et al., 2008; Ferrucci et al., 2010; Ganz et al., 2008; Jason et al., 2001). Therefore, it is important to re-evaluate iron supplementation program for pregnant women since giving iron supplement for non-IDA pregnant women could lead to insulin resistance and cause metabolic syndrome (Bo et al., 2009; Bowers et al., 2011).

5. CONCLUSIONS

Polymorphism of ferroportin gene promoter -1355 G/C was not a risk factor of all anemia in pregnancy, but it was specific for iron deficiency anemia. It was proven by low Hb level and erythocyte indices followed by high sTfR level among pregnant women with C allele variant. This variant caused iron export disturbance which led to IDA. Iron storage was not disturbed since there was no significant correlation between Hb and ferritin level.

ACKNOWLEDGMENT

We would like to thank Risbin IPTEKDOK 2012 Ministry of Health Republic of Indonesia and Universitas Gadjah Mada.

REFERENCES

1. Akesson, A., Bjellerup, P., Berglund, M., Bremme, K., Vahter, M. (1998). “Serum Transferrin Receptor: A Specific Marker of Iron Deficiency in Pregnancy.” Am. J. Clin. Nutr., 68(6), 1241-6.

2. Bo, S., Menato, G., Villois, P., Gambino, R., Cassedar, M., Cotrino, I., Cavallo-Perin, P. (2009). "Iron Supplementation and Gestational Diabetes in Midpregnancy." Am. J. Obstet. Gynecol., 201(2), 158.e1-6.
3. Bowers, K., Yeung, E., Williams, M.A., Qi, L., Tobias, D.K., Hu, F.B., Zhang, C. (2011). "A Prospective Study of Pre-Pregnancy Dietary Iron Intake and Risk for Gestational Diabetes Mellitus." Diabetes Care, 34(7), 1557-63.
4. Crichton, R.R. (2009). Iron Metabolism: From Molecular Mechanism to Clinical Consequences, 3rd Ed., John Willey and Sons, UK.
5. Cui, Y., Wu Q., Zhou, Y. (2009). "Iron-refractory Iron Deficiency Anemia: New Molecular Mechanisms." Kidney Int., 76(11), 1137-41.
6. Farmawati, A., Istiqomah, N., Dewi, V.S., Umarghanies, S.S. (2012). "Anemia Defisiensi Besi pada Ibu Hamil: Analisis Polimorfisme Gen Ferroportin Q248H dan Asupan Nutrisi." Laporan Dana Masyarakat, Fakultas Kedokteran Universitas Gadjah Mada, Yogyakarta.
7. Ferrucci, L., Semba, R.D., Guralnik, J.M., Ershler, W.B., Bandinelli, S., Patel, K.V., Sun, K., Woodman, R.C., Andrews, N.C., Cotter, R.J., Ganz, T., Nemeth, E., Longo, D.L. (2010). "Proinflammatory State, Hepcidin, and Anemia in Older Persons." Blood, 115(18), 3810-6.
8. Ganz, T., Olbina, G., Girelli, D., Nemeth, E., Westerman, M. (2008). "Immunoassay for Human Serum Hepcidin." Blood, 112(10), 4294-7.
9. Hallendorff, M.A. (2008). "Ironing Out Haemochromatosis: A Study of An Indian Family." South Africa: University of Stellenbosch.
10. Jason, J., Archibald, L.K., Nyanyanwu, O.C., Bell, M., Jensen, R.J., Gunter, E., Buchanan, I., Larned, J., Kazembe, P.N., Dobbie, H., Jarvis, W.R. (2001). "The Effects of Iron Deficiency on Lymphocyte Cytokine Production and Activation: Preservation of Hepatic Iron but Not at All Cost." Clin. Exp. Immunol., 126(3), 466-73.
11. Kell, D.B. (2009). "Iron Behaving Badly: Inappropriate Iron Chelation as Major Contributor to the Aethiology of Vascular and Other Progressive Inflammatory and Degenerative Disease." BMC Med. Genomics, 2(2).
12. Knutson, M.D. (2010). "Iron-sensing Proteins that Regulate Hepcidin and Enteric Absorption." Annu. Rev. Nutr., 30, 149-71.
13. Lopes-Sierra, M., Calderon, S., Gomez, J., Pilleux, L. (2012). "Prevalence of Anaemia and Evaluation of Transferrin Receptor (sTfR) in the Diagnosis of Iron Deficiency in the Hospitalized Elderly Patients: Anaemia Clinical Studies in Chile." Anemia, 646201.
14. Lwanga, J.B. (2008). "Serum Ferritin Levels and Birth Outcomes for Rural Pregnant Women in Tororo District." Uganda: Child Health and Development Centre Makarere University.
15. Lymboussaki, A., Pignatti, E., Montosi, G., Garuti, C., Haile, D.J., Pietrangelo, A. "The Role of the Iron Responsive Element in the Control of Ferroportin1/IREG1/MTP1 Gene Expression." J Hepatol., 39(5), 710-5.
16. Mackenzie, E.L., Iwasaki, K., Tsuji, Y. (2008). "Intracellular Iron Transport and Storage: From Molecular Mechanism to Health Implication." Antiox. Red. Sign., 10(6), 997-1030.
17. Ministry of Health Republic of Indonesia. (2005). "Laporan Nasional Riset Kesehatan Dasar 2005." Indonesia: Ministry of Health.
18. Nemeth, E. (2010). "Targeting the Hepcidin-Ferroportin Axis in the Diagnosis and Treatment of Anemias." Adv. Hematol., 2010, 750643.
19. Panton, N. (2008). "Mutation Analysis of Four Genes Implicated in Iron Homeostasis in Porphyria Cutanea Tarda (PCT) Patients." South Africa : University of Stellenbosch.
20. Pietrangelo, A. (2004). "The Ferroportin Disease". Blood Cells. Mol. Dis., 32(1), 131-8.
21. Van der Broek, N.R., Letsky, E.A., White, S.A., Shenkin, A. (1998). "Iron Status in Pregnant Women: Which Measurements are Valid?. Br. J. Haematol., 103(3), 817-24.
22. Wians, F.H., Urban, J.E., Keffer, J.H., Kroft, S.H. (2001). "Discriminating between Iron Deficiency Anemia and Anemia of Chronic Disease Using Traditional Indices of Iron Status VS Transferrin Receptor Concentration." Am. J. Clin. Pathol., 115(1), 112-8.
23. Wish, J.B. (2006). "Assessing Iron Status: Beyond Serum Ferritin and Transferrin Saturation." Clin. J. Am. Soc. Nephrol., 1(Suppl 1), S4-8.

24. World Health Organization. (2008). "Worldwide Prevalence of Anaemia 1993-2005." Geneva: WHO Global Database on Anaemia.